A. Title:

Application for Permit for Scientific Purposes and to Enhance the Propagation or Survival of Listed Species Under the Endangered Species Act of 1973.

B. Species:

Proposed areas of sampling: 1. Icicle Creek, above and below Leavenworth National Fish Hatchery 2. Entiat River above and below Entiat National Fish Hatchery 3. Methow River, above and below Winthrop National Fish Hatchery: spring and summer Chinook, cutthroat, rainbow, and steelhead trout, whitefish, dace, northern pike minnow, and Cottids

C. Date of Permit Application:

10 February 2007

D. Applicant Identity

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E. Information on Personnel, Cooperators, and Sponsors

Principle Investigator: Sonia L. Mumford DVM
Field Supervisors: Dave Carie, Chuck Hamstreet, Dave Zajac, Ray Brunson, Joy Evered, Howard Gearns, Mark Nelson, Barb Kelly-Ringel, Matt Cooper, RD Nelle, Chris Pasley, Al Jensen, Ken Lujan, Laura Kessel, Craig Eaton

Field Personell: Terri Scamahorn, Lance Schott, Rebecca Christopherson, Jason Reeves, John Reier, Bob Adams, Chris Dammon, Craig Chisom, Chris Patterson, Terry, Rod Engle

Proposed Funding source:
US Fish and Wildlife Fons Project
US Fish and Wildlife Service Olympia Fish Health Center Station Funds
Cooperating Institutions: US Fish and Wildlife Service, Mid-Columbia River Fishery Resource Office (MCRFRO) Contact Brian Cates)); US Fish and Wildlife Service, Abernathy Fish Technology Center (Contact Don Campton)

Disposition of Tissue Samples: Head, Kidney, Spleen, Gill, Skin, will be used for fish health sampling. A skin sample will also be taken for genetic testing at the Abernathy Fish Technology Center.

There will be limited transport and no long term holding of these fish. At the completion of transport, the fish will be lethally sampled.

F. Project Description, Purpose and Significance:

The purpose of this project is to gather or continue to gather baseline fish health data for wild fish above and below three National Fish Hatcheries. The most important weapon needed to control or prevent fish disease is knowledge. This information could be extremely important in the management of these stocks. Valuable stocks of fish are possibly at risk because of our lack of knowledge about the distribution of pathogens in wild fish.

In the 1990's the causative agent of whirling disease was associated with a precipitous decline in wild trout in the intermountain west. It focused the Nation's attention on the fact that very little is known about diseases among wild fish. As a result, Congress appropriated funds to the U.S. Fish and Wildlife Service for the National Wild Fish Survey. We are utilizing the funds for this Survey to monitor pathogen distribution above and below our National Fish Hatcheries.

MCRFRO is operating a screw trap in Entiat River to gather information about the fresh water production and life history characteristics of spring Chinook salmon in the Entiat River Basin. By working together, we can lessen potential impacts on collected fish in this area. We will also be exchanging information to enhance both of our studies.

The rational for including in listed species the collection for the survey is that for the last 4 years of collecting at Entiat River and Icicle Creek, we not been successful at finding a "surrogate species" for the listed species. We were hoping to find a species that had similar pathogen profiles at Entiat and Icicle Creek. The most significant pathogen found was *R. salmoninarum*, the causative agent of Bacterial Kidney Disease, which was found in both listed and non-listed species, but not at the same rates. Thus, we are proposing to continue to collecting listed species, although in reduced numbers (60 juvenile Upper Columbia Steelhead Trout, 60 juvenile Upper Columbia Chinook Salmon) at each water. An example where this information would be valuable, if in the future, a pathogen is found to be associated with a large die-off of listed fish, it would be helpful to know whether that pathogen was endemic in that population of wild fish, or if it was introduced.

The fish health sampling that we are proposing is lethal. Currently, accepted diagnostic tests for diseases important to the management of salmonids require lethal sampling. Non-lethal methods are under development, but must be benchmarked and accepted by the fish health community to be considered a valid substitute for lethal sampling.

G. Project Methodology

Duration: April, 2003-November 2006
Procedures and Techniques:

Sampling sites will be selected by the appropriate personnel at the USFWS' Mid-Columbia River Fishery Resource Office. Selections will be based on water flow and conditions at the time of sampling. Sites will be selected at Icicle Creek above and below Leavenworth National Fish Hatchery, Winthrop and Entiat National Fish Hatchery.

Method of Capture: Seine Nets (50' x 4' x 3/16" mesh; 45' x 5' x ¼" mesh; 45' x 4' x 1/8" mesh); Fyke Net (Frame 4' x 4', 1/8" mesh); Live boxes (2' x 2' 4' x 1/8" mesh, 1' x 1' x 1' x 1/8" mesh); electrofishing (backpack and boat mounted) (in accordance with NMFS guidelines); minnow traps; a Screw Trap, provided by MCRFRO, and hook and line.

These nets will be operated by Fish and Wildlife Service employees experienced in fish capture and every attempt will be made to minimize impacts on fish. Electrofishing will be performed by US Fish and Wildlife staff certified in electrofishing techniques.

Fish to be lethally sampled will be euthanized with an overdose of MS222, a bath that is greater than 200 ppm. Fish not used in the project will be released with no exposure to drugs.

Numbers and types of samples to be taken from each individual:

1. If fish collected are >100 mm in length
kidney(2), head (1) , spleen(1), hindgut(1), skin/ fin(1)

2. If fish collected are < 100 mm, but > 60 mm in length
kidney (1), head (1) , spleen(1), hindgut(1), skin/ fin(1)

3. If fish collected are < 60 mm
Entire Fish

On selected fish, samples of skin, eye, liver, spleen, kidney, gill, muscle, heart, gonad, intestinal tract, brain, and swim bladder will be taken for histopathology.

On selected fish gill sample and skin/mucous samples will be taken for identification of external parasites.

Sampling protocol:

Fish will be collected using seine nets, fyke nets, electrofishing, traps, and hook and line. They will be euthanized and sampled within four hours. If the fish is examined for external parasites, a skin scrape and gill clip will

be performed at this time. For fish greater than 100mm: The body cavity will be opened with scissors. Spleen and kidney samples will be taken with forceps in an aseptic manner and placed in labeled sterile tubes for further processing in the lab. The hindgut will be removed and placed in labeled tubes containing saline for later examination. The head will be removed and half of the head placed in one labeled ziplock bag and the other half placed in another labeled bag. Instruments will be disinfected between each fish.

For fish <100 mm, but greater than 60 mm: At the discretion of the Fish Health Biologist, samples will be taken either for virology or bacterial kidney disease testing (since there will not be enough sample to do both). Thus, the samples taken will be either kidney and spleen (virology) or kidney only (bacterial kidney disease). All other procedures are the same.

For fish < 60mm: At the discretion of the Fish Health Biologist, either samples of viscera (virology) and head (whirling disease), or whole body (virology only), will be taken.

The fish to be included in this project will be lethally sampled. Fish not in this project will be handled carefully and released as soon as possible. Nets, traps, and electrofishing equipment will be operated by experienced personnel in order to decrease possible impacts to other fish.

H. Description and Estimates of Take at Icicle Creek and Entiat River:

Intentional Take of Wild Fish for the Wild Fish Health Survey

ESU	Life Stage	Take Action	# of individuals	Unintentional Mortality*	Location	Dates
Upper Columbia River Chinook	juvenile	Intentional Mortality	60**	5	Entiat River	April-November 2009-2014
Upper Columbia River Chinook	juvenile	Intentional Mortality	60	5	Icicle Creek	April-November 2009-2014
Upper Columbia River Chinook	juvenile	Intentional Mortality	60	5	Methow River	April-November 2009-2014
Upper Columbia River Steelhead	juvenile	Intentional Mortality	60	5	Entiat River	April-November 2009-2014
Upper Columbia River	juvenile	Intentional Mortality	60	5	Icicle Creek	April-November 2009-

Steelhead						2014
Upper Columbia River Steelhead	juvenile	Intentional Mortality	60	5	Methow River	April-November 2009-2014

*This is a high estimate of unintentional mortality. This number is based on a five percent capture mortality, since we will be able to use any mortalities that we can find and incorporate them in the intentional mortalities, this number will probably be significantly less. In addition we will be using a variety of collection methods, some of which are associated with a lower capture mortality than 5%. All Chinook collected and sampled will be naturally reproducing fish. The hatchery stock at the hatcheries are not listed species, so are not included in this permit. The actual take on listed Chinook will likely be less than shown above because some of the fish we will sampling are offspring from naturally reproducing hatchery returns (a non-listed stock).

No Adults will be sacrificed for this study.

**These are maximums. Our numbers may drop depending on population estimates (when available) by the US FWS Leavenworth Fisheries Resource Office Staff and our ability to capture fish. Up to 180 whitefish , brook trout (*Salvelinus fontinalis*), summer Chinook(*O. tshawytscha),* and rainbow trout (*O.*mykiss).

Estimates of Unintentional take of Wild/ Hatchery Fish

Unintentional take will be dependent on what collection methods are employed.

Fyke Net	5%
Electroshocking	5%
Seining	1%
Smolt Trap	1%
Hook and Line	1%

Collection methods will be determined by MCRFRO staff based on minimizing impacts to non-target fish and maximizing our ability to catch fish. Electroshocking will be performed by certified staff and in accordance with NMFS guidelines.

Ideally, our primary target population is spring Chinook smolts, since we are comparing them to the hatchery fish which are also spring Chinook. However, since there are not significant numbers of spring Chinook above the hatchery, we will be targeting other salmonids as they will most closely represent what may be occurring in the spring Chinook. In addition, baseline data is needed for all species. Smolts were chosen because they provide the most sample material, in order to have the ability to run as many tests as possible. Although fry could be used, due to inadequate tissue volume, fewer tests could be run. The above take numbers would be for large run years, but they would be adjusted downward if there was a poor run. We would be comparing naturally reproducing fish with those at the hatchery.

The sampling schedule will be dependent on water flows and environmental conditions. We will be sampling above and below the federal hatcheries on the Entiat River and Icicle Creek.

I. Transportation and Holding
N/A

J. Cooperative Breeding Program
N/A

K. Previous or Concurrent Activities Involving Listed Species
N/A

L. Certification
I hereby certify that the foregoing information is complete, true and correct to the best of my knowledge and belief. I understand this information is submitted for the purpose of obtaining a permit under the Endangered Species Act of1973 (ESA) and regulations promulgated thereunder, and that any false statement may subject me to criminal penalties of 18 U.S.C 1001, or to penalties under the ESA."

_____ Date _____

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